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DISJUNCT POPULATIONS OF *TRIFOLIUM BECKWITHII* (FABACEAE) IN EASTERN SOUTH DAKOTA: VICARIANCE OR RECENT LONG-DISTANCE DISPERSAL? A PRELIMINARY ANALYSIS

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ABSTRACT

Trifolium beckwithii Brewer ex. S. Wats. is widespread in the Sierra Nevada and northern Rocky Mountains but five disjunct populations have existed for at least a century in a considerably different habitat in eastern South Dakota. Genetic divergence of these populations was expected. RAPD profiles were compared between South Dakota and two montane populations. Unexpectedly, measures of genetic similarity between South Dakota and northern California populations were twice as great as those between South Dakota and southern Idaho populations. These data can be interpreted as an indication that *T. beckwithii* has been introduced relatively recently into South Dakota from a population genetically similar to the one sampled from northern California.

Trifolium beckwithii Brewer ex. S. Wats. is a low growing, rhizomatous, perennial clover endemic to the western U.S. and commonly known as buffalo or Beckwith's clover. Beckwith's clover is distinguished from congeneric species by spherical heads of subsessile purple or rose-purple flowers and with narrowly elliptic, glabrous leaflets. The species is found primarily in montane meadows of the Sierra Nevada Mountains in northern California and Oregon ranging eastward to the Rocky Mountains of southwestern Montana (Fig. 1). It typically occurs in moist, streamside meadows at elevations of 1,200 to 2,125 meters above mean sea level (msl) (Gillett 1972). Unexpectedly, disjunct populations of this species also occur historically at five sites in extreme eastern South Dakota in moist tallgrass prairies located in the upper Big Sioux River watershed. These sites are at only 500 to 600 meters above msl and about 1200 km east of the nearest population which is in southwestern Montana (Fig. 1). These sites are closely clustered and do not extend into nearby Minnesota (Cholewa personal communication). The earliest report of these disjunct populations extends back a century (Saunders 1899).

To our knowledge no other species of plant has this distribution. The most similar phytogeographical patterns are those of species that occur in the northern Rocky Mountains with disjunctions in the Black Hills of western South Dakota and/or along the Great Lakes, e.g., *Adenocaulon bicolor* Hook, *Habenaria unalascensis,* (Sprengel) S. Watson and *Vaccinium membranaceum* Hook. (Marquis and Voss 1981). Disjunct distributions can be explained in two ways: 1) vicariance; and 2) long distance dispersal. Under the first of these two hypotheses, *T. beckwithii* in eastern South Dakota is a relict of a widespread and relatively old distribution. The habitat of the South Dakota populations of *T. beckwithii* has been significantly different from that of the cordilleran populations for at least several thousand years; a habitat that was frequently burned, periodically grazed by large bison herds, and subjected to irregular periods of drought. Note that the rhizomatous habit of *T. beckwithii* is preadapted to the prairie regime. Under this hypothesis the South Dakota populations, long isolated from the montane populations and environment, would be expected to exhibit substantial divergence when compared with montane populations of the species, and would not be expected to show significantly greater similarity to any one of the montane populations.

Alternatively, the species may have been introduced from the more typical western populations relatively recently. Humans are the most likely agents of dispersal since neither seeds, fruits, nor vegetative cuttings of *T. beckwithii* appear to be harvested by birds or other wide-ranging species. According to this hypothesis, little divergence of *T. beckwithii* in South Dakota would be expected when compared to montane populations, and greater similarity would be observed with the parent population from which the South Dakota populations were derived.

Divergence patterns related to geography might be expected to be reflected in morphological characters. Consequently, 112 herbarium specimens representing both cordilleran and South Dakota populations were measured with respect to the following characters: lengths and widths of stems, petioles, peduncles, leaflets, stipules, and inflorescences; lengths of calyces and calyx teeth, total corolla, wing petals, keel petals, stamens, anthers, styles, pods, and seeds, and finally the number of seeds per fruit. Variation in vegetative features in

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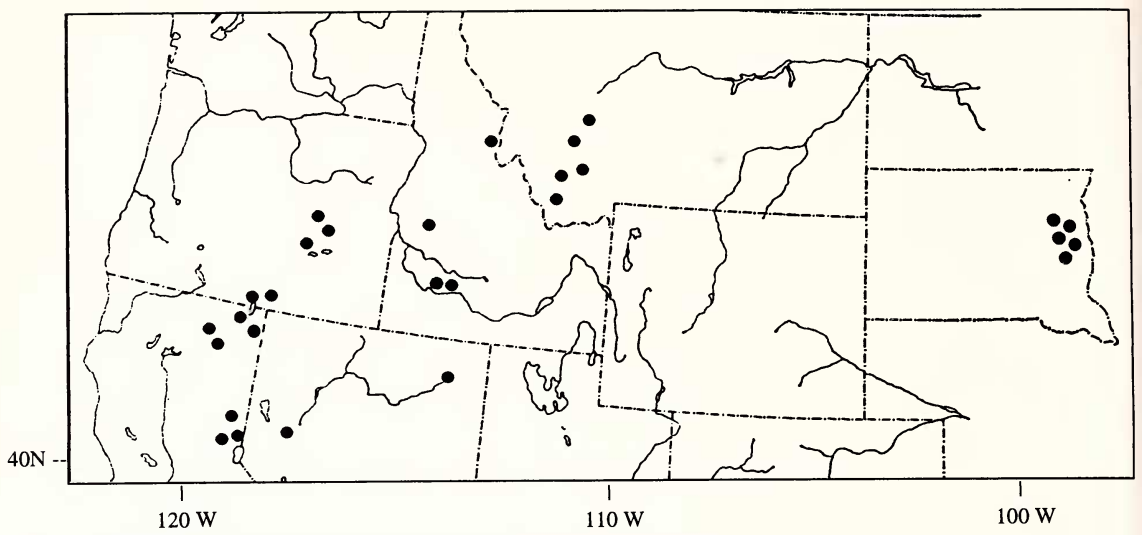

FIG. 1. Distribution of *Trifolium beckwithii,* adapted from Gillett (1972).

particular was considerable but with no correlation between variation in any features and geographic origin (Larson unpublished). Consequently, we chose to investigate genetic differences by assessing divergence in molecular characters.

Our specific objective was to assess the extent of divergence of a disjunct population of *T. beckwithii* in eastern South Dakota from two selected cordilleran populations by analysis of Random Amplified Polymorphic DNA's (RAPD's). The RAPD technique was first described by Williams et al. (1990). RAPD analysis allows the determination of the distribution of dominant DNA markers within populations. These markers are variable within populations of organisms and the technique has been successfully used to assess genetic variation in diverse plant species (reviewed in Newbury and Ford-Lloyd 1993). The RAPD technique does not require prior knowledge of target DNA sequences, an advantage when initiating research on a little-studied species.

MATERIALS AND METHODS

Material of *Trifolium beckwithii* was obtained from five individuals in each of three populations (Table 1). Seeds were obtained from the Western Regional Plant Introduction Station (WRPIS) at Pullman, Washington which had been collected from a site in Sierra County, northern California, near the Nevada border. These seeds were grown into 60-day old plants before harvest. Shoots were collected from a second site in Camas County in southern Idaho and immediately packed with silica gel for delivery to South Dakota State University prior to processing. Fresh shoots were also collected from a sparse population consisting of five individuals, in Rauville prairie in Codington County, South Dakota. Attempts at this same time (June 1997) to locate *Trifolium beckwithii* at two other previously documented sites for the species in South Dakota (Aurora Prairie, Brookings County and Quail Prairie, Deuel County) failed to locate any specimens either suggesting localized extinction or prolonged dormancy due to unseasonably wet and cool conditions.

Nucleic acids were extracted from leaves of each accession by the CTAB method (Doyle and Doyle 1987) and RNase-digested by standard procedures. Final concentrations of extracts were diluted to approximately 50 ng/μl. Sixty-three random decanucleotide primers were obtained for initial screening (Operon Technologies Inc., Alameda, CA). To min-

TABLE 1. COLLECTIONS OF *TRIFOLIUM BECKWITHII* USED FOR RAPD ANALYSIS.

Location	Date	Collectors	Voucher or WRPIS collection number
Sierra County, CA	Aug. 1995	N. Taylor, K. Quesenberry and J. Descries, Western Regional Plant Introduction Station, Pullman	C-107
Camas County, ID	May 1997	J. Smith, Boise State University	J. Smith, 3707
Codington County, SD	June 1997	M. Duvall and G. Larson, South Dakota State University, Brookings	G. Larson, s.n.

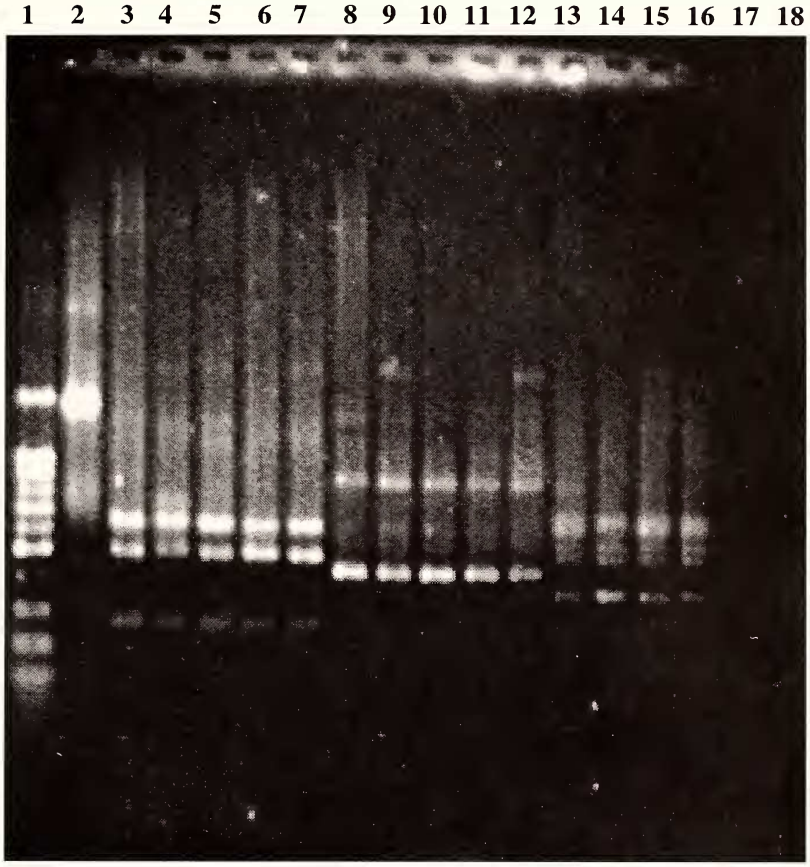

FIG. 2. Representative RAPD profiles (primer, OPB-11) of 15 individual accessions of *Trifolium beckwithii* from three sites after electrophoresis in a 0.8% agarose gel. Lane 1: 100 base pair ladder; Lane 2: amplification control; Lanes 3–7: northern California templates; Lanes 8–12: southern Idaho templates; Lanes 13–17: eastern South Dakota templates (note that the one template in lane 17 failed to amplify, as was typical); Lane 18: negative control (no template).

imize contamination, reactions were prepared in a laminar flow hood with a set of dedicated micropipettors using aerosol resistant tips. RAPD reactions were set up in Promega reaction buffer (final concentration of $MgCl_2$, 1.5 mM) with 0.02 mM dNTPs, 5 pmoles random primer, approximately 20 ng template DNA, and 1.25 units of Taq DNA Polymerase (Promega Corp., Madison, WI). At least one negative control was included with each set of reactions in which sterile water was substituted for the template DNA. Amplifications were performed in a PTC100 thermal cycler (MJ Research, Inc., Watertown, MA). After a hot start (94°C for 2.0 min), 45 amplification cycles of denaturation (94°C for 0.5 min), annealing (30°C for 1 min), and polymerization (72°C for 2 min) were performed with a final extension at 72°C for 10 minutes. Amplified fragments (20 μl of each reaction) were separated electrophoretically on 0.8% agarose gels in TBE, stained with ethidium bromide, visualized under short wavelength UV, and photographed. A 100 base pair ladder (Promega Corp., Madison, WI) provided molecular size markers. A representative photograph of a gel is given (Fig. 2). After the initial screening, primers which produced polymorphic RAPD profiles were run at least one additional time. Bands were observed directly on the photographs of the gels and scored as binary presence/absence data for each template. Jaccard coefficients (Table 2) were calculated for each pairwise comparison of individual accessions as a measure of genetic similarity (Jaccard 1908). These coefficients were selected since negative matches are appropriately excluded from the similarity measure. Mean Jaccard coefficients were calculated for each pairwise population comparison (Table 3). Unweighted pair group method of analysis (UPGMA) cluster analysis (Sokal and Sneath 1973) was performed on the binary data as implemented in PAUP* 4:0 (Swofford 1998, Fig. 3).

RESULTS

Of the 63 original random primers, 30 gave reproducible polymorphic profiles and no amplified

Table 2. Pairwise Jaccard Coefficients by Accession.

	CA2	CA3	CA4	CA5	ID1	ID2	ID3	ID4	ID5	SD1	SD2	SD3	SD4
CA1	0.643	0.695	0.717	0.690	0.160	0.141	0.116	0.123	0.105	0.225	0.230	0.338	0.237
CA2		0.633	0.683	0.600	0.197	0.188	0.175	0.203	0.192	0.258	0.197	0.308	0.203
CA3			0.817	0.793	0.162	0.212	0.200	0.183	0.202	0.246	0.209	0.368	0.197
CA4				0.754	0.157	0.204	0.193	0.176	0.195	0.254	0.200	0.352	0.188
CA5					0.197	0.217	0.190	0.188	0.207	0.235	0.179	0.319	0.167
ID1						0.579	0.589	0.555	0.571	0.159	0.133	0.111	0.119
ID2							0.904	0.678	0.690	0.107	0.083	0.139	0.086
ID3								0.661	0.617	0.093	0.084	0.141	0.087
ID4									0.736	0.132	0.091	0.151	0.094
ID5										0.141	0.101	0.158	0.104
SD1											0.605	0.622	0.639
SD2												0.511	0.636
SD3													0.500

products in the negative controls. One of the five individual DNA extracts from the South Dakota population consistently failed to amplify and eventually this accession was omitted from the analysis. A total of 105 bands were scored from the 30 primers over the remaining 14 accessions. Each accession of *Trifolium beckwithii* had a unique RAPD profile. The number of bands for each primer were: OPA2-6, OPA5-1, OPA7-2, OPA14-3, OPA15-3, OPA16-4, OPA18-4, OPB2-3, OPB6-1, OPB8-5, OPB10-4, OPB11-8, OPB12-3, OPB13-3, OPB14-3, OPB16-5, OPB20-3, OPC3-1, OPC6-3, OPC7-3, OPC8-6, OPC10-6, OPC13-5, OPC19-1, OPD1-2, OPD9-3, OPD12-5, OPD14-2, OPD15-4, and OPD16-3. The raw data matrix may be obtained from the first author on request.

Mean intrapopulational similarity measures were relatively high, as expected, ranging from 0.586 to 0.702 and were not significantly different from each other (Anova; $P < 0.005$). All individuals clustered into their respective populations in the UPGMA analysis (Fig. 3).

Mean similarity measures between populations were highest in the comparison between South Dakota and California (0.246), intermediate between the two montane populations (0.179), and lowest between South Dakota and Idaho (0.116 Table 3). Mean pairwise interpopulational similarity measures were, in every case, significantly different from each other (Anova; $P < 0.001$). The South Dakota accessions clustered with the northern California accessions in the UPGMA analysis (Fig. 3).

Discussion

The distribution of *Trifolium beckwithii* is uniquely different from that of thousands of other plant species found in the western U.S. Such a distribution may suggest a similarly unique underlying origin. Perhaps the first attempt to explain this disjunct pattern was by a doctoral student working on a revision of native U.S. *Trifolium* spp. who suggested that the eastern populations had been introduced from the west (Martin 1943). Gillett (1972) disagreed because the most distantly separated South Dakota populations are nearly 100 km apart and the five individual sites are relatively isolated from each other necessitating either five separate introductions from farther west or a series of regional movements after the original introduction. Furthermore, *T. beckwithii* in eastern South Dakota is restricted to pristine native prairie habitats, a characteristic not expected of a recently introduced species. In either case no likely agent of dispersal is known.

Although Gillett did not propose an alternative explanation, a logical possibility is vicariance. Perhaps *T. beckwithii* in South Dakota was once part of a continuous range which originally extended across the state and into Montana, most likely during the retreat of Wisconsin glaciation (ca. 15,000 to 10,000 years ago), and has contracted into its current distribution because of unfavorable climatic changes or other factors. Note that *T. beckwithii* is preferred as forage by livestock (Larson and Duvall personal observation)

Table 3. Mean Pairwise Jaccard Coefficients of Genetic Similarity by Population.

	Montane, northern California	Montane, southern Idaho	Prairie, eastern South Dakota
Montane, northern California	0.702	0.179	0.246
Montane, southern Idaho		0.658	0.116
Prairie, eastern South Dakota			0.586

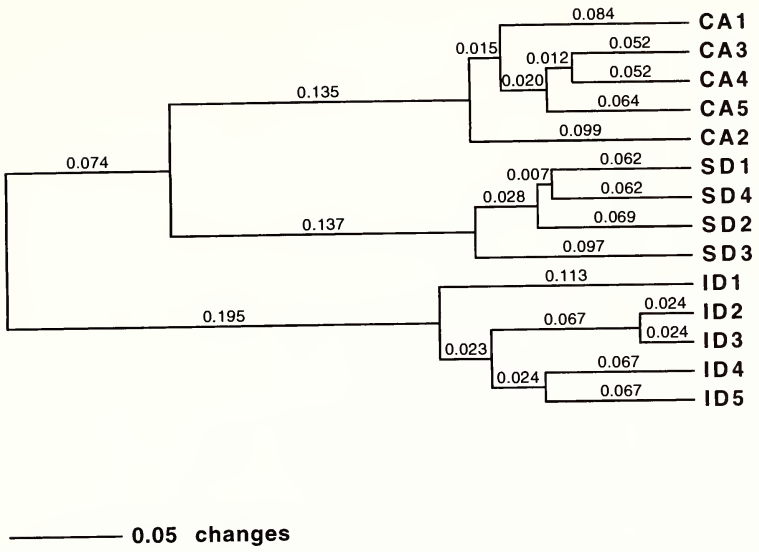

FIG. 3. UPGMA cluster analysis of 105 RAPD loci from 14 accessions of *Trifolium beckwithii* collected from three sites. Labels indicate the site of origin of each individual; CA1–5: northern California site; ID1–5: southern Idaho site; SD1–4: eastern South Dakota site. Branch lengths are given as calculated from simple matching coefficients. The scale bar represents a branch length of 0.05.

and perhaps by other grazing animals. Under this scenario, the implication is that *T. beckwithii,* the only clover native to South Dakota, should be considered endangered in the state, especially given evidence of its recent decline, and state conservation efforts and resources should be expended on behalf of this regionally rare species.

Paradoxically, the RAPDs data presented here do not support the vicariance scenario. Mean genetic measures among the three widely separated populations show a remarkable pattern. The eastern South Dakota and northern California populations have over twice the genetic similarity of the South Dakota and southern Idaho populations which are geographically nearer. Recall that this result has strong statistical support (interpopulational means are significantly different at $P < 0.001$). These data thus appear to support an original dispersal event, sometime prior to 1899, from a parent population genetically similar to the one sampled here from northern California. Given the isolation of South Dakota populations from cordilleran populations and the propensity for vegetative reproduction in *T. beckwithii,* preservation of the original genetic profile over the last century is likely. Note that genetic variation within each of the three populations sampled here is not significantly different suggesting that the propagation mechanisms which affect genetic diversity in the species are not geographically dependent.

These data thus suggest that *T. beckwithii* is a relatively recent immigrant to eastern South Dakota, and neither morphological nor molecular evidence indicate taxonomic distinction of the South Dakota isolate. However, given our limited sample size (five individuals in each of only three populations), recommendations for conservation measures at the federal level based on only these data would be premature and require additional populational analysis.

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